EFFECT OF CHRONIC D-FENFLURAMINE ADMINISTRATION ON RAT BRAIN SEROTONIN LEVELS AND RELEASE

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ABSTRACT. We have applied ex vivo and in vivo methods to examine the possible effects of chronic d-fenfluramine administration on rat brain serotonin (5-HT) levels and release. Low anorectic doses of d-fenfluramine (1.25 - 5 mg/kg/day for 10 days) did not change hypothalamic 5-HT levels or release (basal and electrically-evoked) 6 days after the last treatment. Repeated administration of a higher dose of d-fenfluramine (10 mg/kg/day) reduced hypothalamic and frontal cortex levels of 5-HT. Evoked 5-HT release was proportionately decreased in both brain areas, but basal 5-HT release was unaffected. Administering a dose of tryptophan restored evoked 5-HT release in the frontal cortex. These data suggest that only at doses which far exceed those needed to inhibit of food intake does d-fenfluramine cause a prolonged reduction in brain 5-HT levels and release. These decrements may result in part from tryptophan depletion within serotoninergic nerve terminals.

INTRODUCTION

Adminstration of a high dose of dl-fenfluramine (≥ 15 mg/kg) (1-3) or d-fenfluramine (6 mg/kg/day) (4) produces a prolonged depletion in rat brain 5-HT levels. 5-HT immunoreactivity is reduced in the brains of rats treated repeatedly with a higher dl-fenfluramine dose (24 mg/kg/day for 4 days) (5). These fenfluramine doses produce elevations in brain d-fenfluramine (the anorexigenic isomer) which far exceed those caused by doses needed to inhibit food intake (0.06 - 1.25 mg/kg d-fenfluramine) (6). Low anorexigenic doses of d-fenfluramine, administered either acutely (1.25 - 2.5 mg/kg) (7) or chronically (3 mg/kg/day for 6 days) (4) do not deplete brain 5-HT levels. We have applied ex vivo and in vivo methods to address the possible effect of chronic d-fenfluramine treatment on the ability of serotoninergic neurons to release their neurotransmitter.

MATERIALS AND METHODS

Male Sprague-Dawley rats were housed in pairs and provided with standard rat chow (26.3% protein) and tap water ad libitum.

Ex Vivo Experiments

Rats were treated daily with d-fenfluramine hydrochloride dissolved in 0.9% NaCl (1.25, 2.5, 5 or 10 mg/kg, intraperitoneally) or its vehicle for 10 days. Six days following the last treatment, hypothalamic tissue was taken and slices were prepared. The slices were superfused, and fractions were collected during rest and with electrical field-stimulation (5 Hz, 2 ms, 100 mA/cm^2, 1400 bipolar pulses). High performance liquid chromatography with electrochemical detection was used to assay 5-HT levels and 5-HT released into the superfusion medium.

In Vivo Experiments

Rats were treated with d-fenfluramine hydrochloride dissolved in 0.9% NaCl (10 mg/kg, intraperitoneally) or its vehicle for 8 days. The next day, the rats were anesthetized and prepared for microdialysis sampling in the frontal cortex. Dialysate fractions were collected under basal conditions or evoked by another injection of d-fenfluramine (10 mg/kg, intraperitoneally). Some rats received a tryptophan dose (100 mg/kg, intraperitoneally) 2 hours prior to monitoring basal 5-HT release and the release evoked by an acute d-fenfluramine challenge. Frontal cortex tissue was retrieved at the end of each experiment. 5-HT levels in the tissue and dialysate medium were assayed by high performance liquid chromatography with coulometric detection; tissue tryptophan levels were assayed fluorimetrically.

RESULTS

Ex Vivo Experiments

Hypothalamic 5-HT contents were unchanged in rats treated with 1.25 - 5 mg/kg/day of d-fenfluramine, but were reduced by 22 ± 4% ($p < 0.01$) at the highest drug dose tested (10 mg/kg/day) (Table 1).

Spontaneous 5-HT release was unaffected by any of the d-fenfluramine doses tested, and 5-HT release evoked by electrical field-stimulation was unchanged after d-fenfluramine doses of 1.25 - 5 mg/kg/day. Slices prepared from rats which received 10 mg/kg/day of d-fenfluramine reduced their rate of electrically-evoked release by 20 ± 5% ($p < 0.05$).

In Vivo Experiments

Frontal cortex tryptophan levels were reduced by 60% ($p < 0.05$) following chronic d-fenfluramine (10 mg/kg/day) administration; tissue 5-HT content was reduced by 69% ($p < 0.05$) (Table 2). Fenfluramine-evoked 5-HT release was also diminished (by 82%, $p < 0.05$) by the chronic drug treatment, but basal 5-HT release was unaffected. Administering tryptophan (100 mg/kg, intraperitoneally) prior to the acute d-fenfluramine challenge restored frontal cortex tryptophan levels and evoked 5-HT release; tissue 5-HT stores were partially replenished.

TABLE 1. Effect of chronic d-fenfluramine administration on hypothalamic 5-HT levels and ex vivo release

	D-Fenfluramine Dose (mg/kg/day)			
	1.25	2.5	5.0	10.0
Tissue Levels				
vehicle	79±5	76±8	85±7	84±5
drug	78±7	72±6	72±5	66±6**
%	98±6	97±7	86±6	78±4
Basal Release				
vehicle	55±6	50±8	57±4	55±6
drug	49±8	51±5	53±3	54±8
%	87±6	108±10	93±4	100±15
Evoked Release				
vehicle	285±30	239±25	290±30	301±28
drug	274±30	261±30	267±34	241±26*
%	96±4	110±6	92±8	80±5

Rats received d-fenfluramine in 0.9% NaCl (drug) or 0.9% NaCl (vehicle) intraperitoneally for 10 days; 6 days following the last day of treatment hypothalamic tissue was taken and slices prepared. Tissue 5-HT levels are expressed as nmol/g protein; amounts of 5-HT release are expressed as pmol/g protein/min. Values are given as group means ± s.e.m. for N = 5 - 8 pairs. * $p < 0.05$, ** $p < 0.01$ differs from vehicle-treated group by Student's paired t-test.

DISCUSSION

These data indicate that chronic administation of d-fenfluramine to rats, even in doses which are considerably higher than those needed to inhibit food intake (1.25 - 5 mg/kg/day), does not cause long-lasting reductions in hypothalamic 5-HT levels. D-fenfluramine given in this dose range also does not diminish the spontaneous and electrically-evoked release of 5-HT from the rat hypothalamus. Repeated injections of an even higher dose (10 mg/kg/day) can cause a prolonged reduction in hypothalamic 5-HT (by 22% six days after the end of treatment) and frontal cortex 5-HT (by 69% one day after the end of treatment), but these levels are back to normal after 28 days (submitted for publication). Despite these reductions in tissue 5-HT content, basal 5-HT release is not altered as measured ex vivo from hypothalamic slices or in vivo by microdialysis in the frontal cortex; evoked 5-HT release is decreased in parallel to the reductions in tissue 5-HT levels. The proportionate reductions in tissue 5-HT content and evoked 5-HT release are reminiscent of the parallel relationship previously observed after tryptophan supplementation increased hypothalamic 5-HT levels (8). This suggests that reductions, as well as elevations, in brain 5-HT levels result in proportionate changes in evoked 5-HT release. And corollary to this, the drug-induced decrements in evoked 5-HT release may

TABLE 2. Effect of a tryptophan dose on chronic d-fenfluramine-induced reductions in frontal cortex 5-HT levels and in vivo release

	Treatment		
	Vehicle	Drug	Drug + Trp
Tissue Levels	14±3	4*	7±1*
Basal Release	20±2	18±1	16±1
Evoked Release	810±380	146±9*	730±220

Rats received 10 mg/kg/day of d-fenfluramine in 0.9% NaCl (drug) or 0.9% NaCl (vehicle) intraperitoneally for 8 days. On the following day a microdialysis probe was implanted in the frontal cortex; basal and acute d-fenfluramine-evoked (10 mg/kg, intraperitoneally) 5-HT release were monitored with or without prior tryptophan (100 mg/kg, intraperitoneally) administration. Tissue 5-HT levels are expressed as nmol/g protein. Basal release is expressed as fmol/15 min dialysate sample and evoked release is given as the peak percent change from basal release. Values are means ± s.e.m. for N = 5 per group. * $p < 0.05$ from vehicle-treated by Dunnett's test following one-way analysis of variance.

be caused by local depletions in tryptophan availability. This hypothetical mechanism to account for the reductions in brain 5-HT levels and release is analogous to the tyrosine depletion and consequent decrease in dopamine release that occurs when superfused striatal slices are electrically stimulated for prolonged periods (9). Support for this hypothesis comes from the present observations that frontal cortex tryptophan and 5-HT levels are reduced following chronic exposure to d-fenfluramine megadoses, and that tissue tryptophan levels and evoked 5-HT release are quickly restored in rats given supplemental tryptophan.

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